1 April 1993 Reprint

Human Volunteer Studies with Campylobacter jejuni

Army Project Order 90PP0820

Robert E. Black, Daniel Perlman, Mary Lou Clements, Myron M. Levine, and Martin J. Blaser

Veterans Administration Medical Center
Research Service
1310 24th Avenue South
Nashville, Tennessee 37212-2637

U.S. Army Medical Research & Development Command
Fort Detrick
Frederick, Maryland 21702-5012

Title of Project Order: Studies of the Outer Membrane Proteins of Campylobacter Jejuni for Vaccine Development

Approved for public release; distribution unlimited

Campylobacter, Vaccines, Biotechnology, ID, Diseases, RAD I, Lab Animals, Volunteers

Unclassified Unclassified Unclassified Unlimited

CHAPTER 23

Human Volunteer Studies with *Campylobacter jejuni*

ROBERT E. BLACK, DANIEL PERLMAN, MARY LOU CLEMENTS, MYRON M. LEVINE, AND MARTIN J. BLASER

INTRODUCTION

Campylobacter jejuni and closely related organisms are established enteropathogens, resulting in diarrhea and often dysentery in susceptible children and adults (6). These organisms also cause asymptomatic infections, which are especially frequent in children residing in developing countries, who appear to be exposed frequently from an early age.

As has been found with other enteropathogens, volunteer studies in healthy adults can provide important insights into the pathogenesis of these illnesses, into the systemic and local immune responses to infection and illness, and into the extent and duration of protection afforded by prior exposure. They can also provide an efficient means of evaluating the reactogenicity, immunogenicity, and efficacy of candidate vaccines and of relating this efficacy to levels of natural protection demonstrated through volunteer rechallenge studies. Thus, studies were initiated to establish a volunteer model of *C. jejuni* diarrhea, including establishing the number of organisms needed to cause illness and the pathogenicity of different strains, and to use this model to evaluate the immune responses following infection and the extent of protective immunity.

ESTABLISHMENT OF THE VOLUNTEER MODEL

Volunteers for these studies were healthy, young adults from the Baltimore community. The methods of medical screening, informed consent, and therapy of illness have been published previously (8). The specific methods used for these studies at the Center for Vaccine Development have also been published (1). Briefly, volunteers were admitted to the isolation ward and challenged with *C. jejuni* suspended in 150 ml of milk (reconstituted from dry skim-milk powder). Four volunteers received the *C. jejuni* with 2 g of sodium bicarbonate instead of milk. After challenge, volunteers were interviewed daily for 12 days by a physician. All stools were collected, graded for consistency, and weighed. During the first 7 days after challenge, diarrhea was defined as the passage of two or more liquid stools, weighing in total at least 200 g, in a period of 48 h, or of a single liquid stool weighing at least 300 g or containing blood. Temperatures were taken every 6 h. Illness was defined as the presence of diarrhea or fever (temperature, >38°C). After the 7-day period of observation, all volunteers were treated with 250 mg of erythromycin stearate every 6 h for 5 days.

Two *C. jejuni* strains were used in these challenge studies. Strain A3249 (Penner serotype 27) was isolated from a 16-year-old boy with a sporadic infection after an outbreak at a camp in Connecticut. The patient had had a 2-day illness with several loose stools, headache, nausea, and a temperature of 38°C. Strain 81-176 (Penner serotype 23/36) was isolated from an ill 9-year-old girl in a milk-borne outbreak in Minnesota. Strain A4349 manifested two colony types, spreading and nonspreading, which were

Robert E. Black and Mary Lou Clements, Department of International Health, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland 21205. **Daniel Perlman,** University Park Medical Clinic, Denver, Colorado. **Myron M. Levine,** Center for Vaccine Development, Division of Geographic Medicine, Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland 21201. **Martin J. Blaser,** Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-2605, and Infectious Disease Section, Department of Veterans Affairs Medical Center, Nashville, Tennessee 37203.

TABLE 1. Results of volunteer studies with *C. jejuni* A3249

C. jejuni challenge dose (CFU)	No. of volunteers	% Infected	% Ill	Mean no. of diarrheal stools	Mean diarrheal vol (ml)
8×10^2	10	50	10	2.0	106
8×10^3	10	60	10	4.0	158
9×10^4	13	85	46	5.3	533
8×10^5	11	73	9	4.0	302
1×10^6	19	79	11	16.0	1,574
1×10^8	5	100	0	2.5	388

subsequently demonstrated to represent flagellated and aflagellated variants, respectively (9). In total, each strain had been passaged 5 to 10 times on artificial media before being given to volunteers; stock cultures of *C. jejuni* for all volunteer studies were stored at −70°C in glycerol. Challenge inocula were prepared as previously described, and replicate pour plate quantitative cultures of the inocula were made before and after challenge to confirm the concentration of viable organisms. Because strain A3249 manifested both spreading and nonspreading colony types, separate challenge inocula were prepared to equally represent these two types in the final inoculum.

Cultures were made of samples of all stools passed after challenge until discharge from the study. If no stool was passed in a 24-h period, a rectal swab was obtained for culture. A wet mount of each stool sample was made on a glass slide and stained with methylene blue, and the number of leukocytes per high-power field was counted by an experienced technician who did not know the clinical status of the volunteers; stools were also tested for blood by using Hemoccult (SmithKline Beckman, Sunnyvale, Calif.). Cultures of blood were done for all volunteers at 0.5, 12, 24, 48, 72, 96, 120, and 144 h after challenge. Jejunal fluid for culture was collected by having volunteers ingest a gelatin string device (Entero-Test; Health Development, Palo Alto, Calif.) at intervals of 20 and 44 h after challenge and withdrawing the string for culture after 4 h. Stool specimens, rectal swabs, and fluid from Entero-Test strings were plated onto Campy-BAP (BBL Microbiology Systems, Cockeysville, Md.) as previously described. Blood cultures in thioglycolate broth were done by two methods, with subsequent incubation in an anaerobic jar with Campy Pak II (BBL Microbiology Systems) and in aerobic and anaerobic BACTEC bottles (BACTEC Systems; Johnson Laboratories, Cockeysville, Md.), and processed by standard methods.

Six studies were performed to establish the relationship between the ingested dose of *C. jejuni* A3249 and the rates of infection and illness (Table 1). At the lowest dose (800 total or 400 flagellated *C. jejuni* organisms), 5 of 10 volunteers became infected and one developed a mild illness. As the inoculum was raised to 10^8 CFU, the rate of infection increased to 100%. With higher doses, the attack rate did not increase consistently, nor did the severity of illness appear to differ. Overall, 11 (15%) of 68 persons given strain A3249 in milk became ill; 10 had diarrhea (4 also had fever), and 1 had fever without diarrhea. Of the four volunteers who received 10^8 CFU with 2 g of sodium bicarbonate, two developed diarrhea. For all volunteers, the incubation period until the onset of fever was 68 h, and the period to the onset of diarrhea was 88.5 h. In general, the diarrhea was mild with an average of approximately five liquid stools and 0.5 liter total stool volume. Anorexia, malaise, and abdominal cramps were reported by 52 to 60% of ill volunteers. All of the volunteers with diarrhea had fecal leukocytes, and the majority had

TABLE 2. Results of volunteer studies with *C. jejuni* 81-176

C. jejuni challenge dose (CFU)	No. of volunteers	% Infected	% Ill	Mean no. of diarrheal stools	Mean diarrheal vol (ml)
1×10^6	7	100	43	29.7	2,896
2×10^8	10	100	60	11.0	1,092
2×10^9	22	100	41	12.3	1,275

TABLE 3. Homologous rechallenge with *C. jejuni* A3249 and 81-176

C. jejuni strain	Volunteer status	Rechallenge dose (CFU)	No. of volunteers		
			Studied	Infected	Ill
A3249	Ill after challenge with 10^8 CFU of *C. jejuni* 1 mo earlier	10^8	2	0[a]	0
	Healthy (control)	10^8	5	5[a]	0
81-176	Ill after challenge with 10^8–10^9 CFU of *C. jejuni* 1 mo earlier	10^9	7	5	0[b]
	Healthy (control)	10^9	12	12	6[b]

[a] $P = 0.048$.
[b] $P = 0.034$.

fecal blood, at least by Hemoccult testing. Sigmoidoscopy in three ill volunteers showed normal rectal mucosa in two, whereas one demonstrated a diffusely abnormal mucosa with edema and loss of the normal vascular pattern. This person and one of those with a normal sigmoidoscopic examination had an abnormal mucosa visualized on a microscopic study of rectal biopsy specimens. These two biopsies showed a mixed population of inflammatory cells with neutrophils in the crypts and lymphocytes and plasma cells in the muscularis mucosa.

Three studies were done with *C. jejuni* 81-176 (Table 2). All volunteers who ingested inocula ranging from 10^6 to 10^9 CFU developed a positive stool culture. Overall, 18 (46%) of 39 volunteers challenged with strain 81-176 became ill with diarrhea (6 also had fever). No obvious dose-response relationship was noted, but the attack rate appeared to be higher than that after challenge with strain A3249, when only 2 (8%) of 24 volunteers receiving 10^6 to 10^8 CFU of *C. jejuni* in milk became ill. The incubation period to the onset of fever was 67 h, and the period to the onset of diarrhea was 53 h. Of the 18 persons who became ill after receiving strain 81-176, the average diarrhea consisted of 15 liquid stools and 1.5 liters of stool volume, illnesses that were substantially more severe than those caused by strain A3249. Anorexia, malaise, and abdominal cramps were reported in 67 to 78% of ill volunteers. All ill volunteers had fecal leukocytes, and 78% had blood in the stool.

In studies with both strains, stool cultures usually became positive by day 2 to 3 after challenge and stayed positive until 24 to 48 h after erythromycin treatment was begun. All infections were eliminated by erythromycin treatment, and no relapses were observed. Despite inoculation with equal numbers of organisms of the spreading and nonspreading colony types of strain A3249, stool cultures revealed only the spreading-colony type. At the high challenge inocula, string cultures of jejunal contents were often positive at 24 h, but rarely at 48 h (1). None of the blood cultures were positive.

These studies demonstrate that ingestion of even low doses of *C. jejuni* results in diarrhea with fecal leukocytes and blood, similar to naturally occurring illness caused by this enteropathogen. Of the two strains used, one produced a higher attack rate and more definite illness, making it a more suitable challenge strain for a volunteer model. The lack of bacteremia during infection and the ability to terminate illness and infection by antibiotic therapy are desirable characteristics in a volunteer model, indicating low risk of serious systemic infection and of relapse or transmission of infection upon return to the community.

PROTECTION INDUCED BY *C. JEJUNI* ILLNESS

Protective immunity by prior disease was evaluated in homologous rechallenge studies

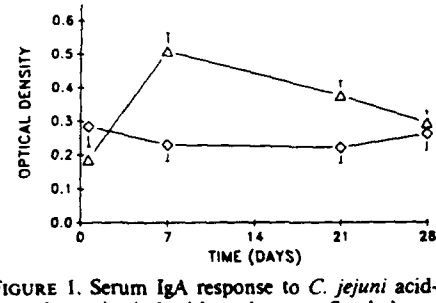

FIGURE 1. Serum IgA response to *C. jejuni* acid-extracted proteins in healthy volunteers. Symbols: △, infected; ◇, not infected.

with each of the strains (Table 3). Two volunteers, who had developed illness after challenge with 10^4 CFU of *C. jejuni* A3249, were rechallenged 28 days later with 10^8 CFU of the same strain, as were with five volunteers who had not participated in previous *C. jejuni* studies. Neither of the two rechallenged persons became infected, whereas all five controls became infected, although none became ill. Seven volunteers who had developed illness after receiving 10^8 to 10^9 CFU of *C. jejuni* 81-176 were rechallenged 28 days later with 10^9 CFU of the same strain, as were 12 controls. Infection occurred in five of the seven previously ill volunteers and in all of the controls. However, diarrheal illness was not observed in previously ill persons on rechallenge, but was observed in 6 of the 12 controls.

These studies indicate that homologous immunity can be induced, at least for the short term, by symptomatic *C. jejuni* infection. This confirms the suggestion from epidemiologic studies that natural infection confers protective immunity and may explain the age-related decrease in the case-to-infection ratio for *C. jejuni* in developing countries (7). The extent and mechanism of heterologous immunity remain to be determined.

IMMUNOLOGIC RESPONSE AFTER CHALLENGE WITH *C. JEJUNI*

Serum samples were collected before challenge and at 7, 21, and 28 days later. Among persons rechallenged on day 28, serum samples were also obtained on days 35, 49, and 56. Jejunal fluid was collected by intubation before and 11 days following challenge or rechallenge with *C. jejuni*. Jejunal aspirate specimens were standardized to 6 μg of total immunoglobulin A (IgA) per well.

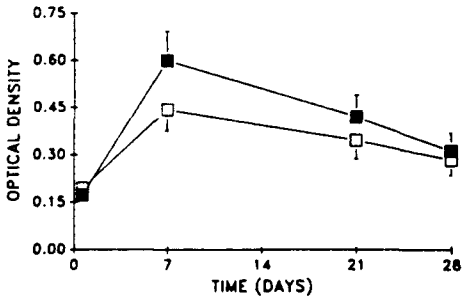

FIGURE 2. Serum IgA response to *C. jejuni* acid-extracted proteins in well (□) and ill (■) infected volunteers.

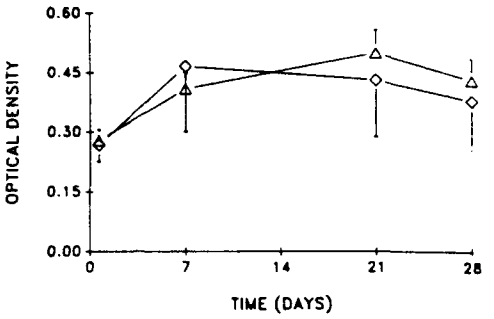

FIGURE 3. Serum IgM response to *C. jejuni* acid-extracted proteins in well (△) and ill (◇) infected volunteers.

Immune Responses to Acid-Extracted Proteins of *C. jejuni*

For the first set of immunologic studies, an enzyme-linked immunosorbent assay (ELISA) with *C. jejuni* group-specific proteins as the antigen was used. The antigen was prepared from Penner 1, 2, and 3 strains by using an acid extraction procedure as has been described previously (2). Goat anti-human Ig class-specific peroxidase conjugates were used in the assay. Because analysis of serially diluted test sera has shown a linear relation between the optical density value and the reciprocal titer, all sera were assayed at single screening dilutions: 1:50 for IgA and IgM and 1:100 for IgG.

Volunteers who were challenged but did not become infected had a higher preexisting IgA level in serum on the day of challenge than those who did become infected ($P = 0.04$) (Fig. 1). Volunteers who did not become infected showed no significant increase in IgA antibody levels following challenge. Infected volunteers had an IgA response that peaked at day 7 and declined to the baseline level by day 28. Rises in antibody levels of IgG and IgM were also noted, but did not differ in infected and noninfected volunteers.

Among volunteers who became infected, those who remained well and those who became ill were compared (Fig. 2). In both groups, the IgA level in serum peaked at day 7, but the ill group had a significantly higher peak IgA level. IgM (Fig. 3) and IgG (Fig. 4) levels peaked at day 21, but did not differ in ill and well volunteers.

Since the severity of illness differed with the two strains evaluated, the serum IgA response was evaluated separately for volunteers given each of the two strains. The serum IgA response was higher after ingestion of strain 81-176 than

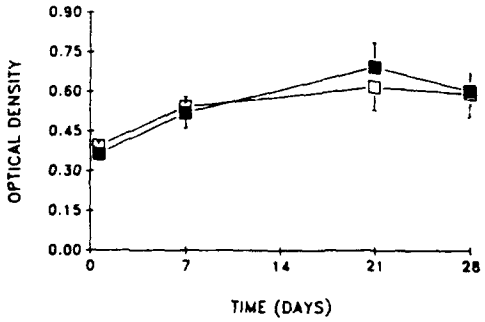

FIGURE 4. Serum IgG response to *C. jejuni* acid-extracted proteins in well (□) and ill (■) infected volunteers.

after exposure to strain A3249 (Fig. 5). Similar differences were seen in IgM and IgG responses.

The immunologic responses of the nine volunteers who became ill and were subsequently rechallenged with homologous strains were studied separately. During the initial illness in these nine volunteers, levels of all three Ig classes rose and had begun to fall by the day of rechallenge on day 28 (Fig. 6). However, the levels of all three Igs were significantly higher at day 28 than they had been prior to challenge. Following rechallenge, there were no additional rises in antibody levels in serum.

Jejunal fluid aspirates were also assessed for *C. jejuni*-specific IgA. The prechallenge levels of intestinal IgA were higher in the group that remained well after challenge than they were in the group that developed illness (Fig. 7), but this difference was not significant ($P > 0.05$). The groups of ill and well volunteers had comparable rises in intestinal IgA levels after challenge. Of the 32 ill volunteers, 9 were rechallenged with the homologous strain of *C. jejuni* on day 28 after the initial challenge. In these volunteers, the levels of intestinal IgA rose on day 11 after challenge and further by day 28. At that time, the IgA levels were similar to the prechallenge levels in the group of volunteers who remained well after the initial challenge. None of the nine rechallenged volunteers became ill, but they did have a further rise in intestinal IgA levels.

Immune Responses to *C. jejuni* LPS

ELISA was used to assay for antilipopolysaccharide (anti-LPS) antibody; serum specimens were evaluated for levels of IgA, IgG, and IgM, and jejunal fluid was evaluated for IgA. The antigen used was prepared from strain 81-176 as described by Blaser and Perez-Perez (this volume). The 2-keto-3-deoxyoctanoate concentration in the phenol-water-extracted LPS preparation was 5.7%, and the protein contamination was 0.87%. By sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 15% acrylamide, silver stain resolved a single band for the LPS preparation. For the ELISA, 1.25 μg of the LPS preparation was used to coat each microtiter plate well. After blocking the plates, 100 μl of serum was placed in each well in triplicate in the following dilutions: IgA, 1:50; IgG, 1:100; IgM, 1:200. Peroxidase conjugates of class-specific goat anti-human Igs (Tago, Burlingame, Calif.) were used, and optical densities were assessed using a Titertek Multiscan (Dynatech Laboratories, Alexandria, Va.). Seroconversion was defined as a rise in optical density of >0.2 U from the prechallenge serum sample in any of the 7-, 21-, or 28-day serum samples. The optical density threshold value was defined to be more than 3 standard deviations from the mean optical density of 25 prechallenge serum samples.

Of the 111 volunteers, 39 were fed *C. jejuni* 81-176, from which the ELISA antigen was derived (homologous group), and 72 volunteers were fed strain A3249 (heterologous group). In 18 of the 39 volunteers in the homologous challenge group, there were 7 IgA, 10 IgG, and 8 IgM seroconversions (Table 4). Of the 18 volunteers who seroconverted in the homologous group, 8 (44%) seroconverted in two Ig classes. All of these eight double seroconverters had a rise in IgG levels, while four each had a rise in IgA and IgM levels. In 18 of the 72 volunteers challenged with A3249 and tested with the heterologous antigen, there were 11 IgA, 6 IgG, and 4 IgM seroconversions. Only 4 (22%) of the 18 volunteers who seroconverted in this group converted in two Ig classes. One of these four volunteers con-

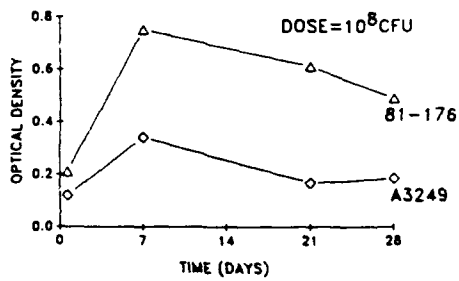

FIGURE 5. Serum IgA response to *C. jejuni* acid-extracted proteins in all volunteers ingesting strains A3249 and 81-176.

TABLE 4. Seroconversion to *Campylobacter* LPS[a] in 111 volunteers challenged with *C. jejuni* strains

Dose (CFU)	No. of volunteers	No. (%) of volunteers with seroconversion		
		IgA	IgG	IgM
Strain 81-176				
1×10^6	7	2 (28.6)	4 (57.1)	2 (28.6)
1×10^8	10	1 (10.0)	1 (10.0)	1 (10.0)
1×10^9	22	4 (18.2)	5 (22.7)	5 (22.7)
Total	39	7 (17.9)	10 (25.6)	8 (20.5)
Strain A3249				
$0.4–5.0 \times 10^4$	33	5 (15.1)	5 (15.1)	2 (6.1)
$0.8–1.0 \times 10^6$	30	4 (13.3)	0 (0)	2 (6.7)
1.0×10^8	9	2 (22.2)	1 (11.1)	0 (0)
Total	72	11 (15.3)	6 (8.3)	4 (5.5)

[a] Derived from strain 81-176.

verted in all three classes. The rate of seroconversion was significantly greater in the homologous group than in the heterologous group ($P < 0.05$), as was the rate of seroconversion to two Ig classes ($P < 0.05$). However, for both groups the seroconversion rates were not dose dependent.

The mean optical densities for the IgA (Fig. 8), IgG, and IgM assays were compared for the homologous and heterologous groups. For IgA, there were no significant differences between the mean optical densities at any sampling point for the two groups of volunteers; however, the homologous group had a consistently higher mean optical density at each datum point. In both groups, the IgA level peaked at day 7 postchallenge and declined to the baseline value by day 28. Neither group of volunteers demonstrated a serologic response assessed by mean optical density in IgM or IgG.

Volunteers who ingested strain 81-176 were divided into two groups: those who became ill and those who remained well (Fig. 9). The ill group had a significant increase in specific IgA levels which peaked at day 7 and dropped to baseline by day 28. The well group, all of whom were infected with *C. jejuni*, had a much smaller rise in IgA levels at day 7 ($P = 0.01$). Mean levels of their IgA antibody did not increase significantly. In volunteers fed the homologous strain, the severity of illness was rated on a scale of 0 to 5, based on the quantity and consistency of diarrheal stools, fever, and associated symptoms. The volunteers were stratified by serologic response to determine whether there was an association between disease severity and serologic

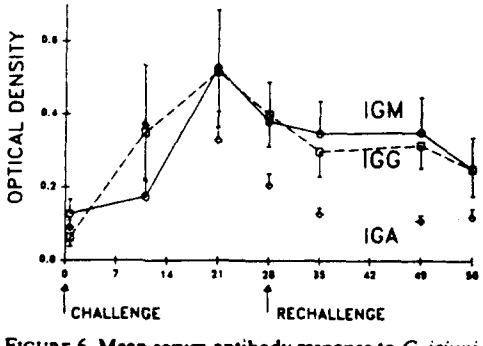

FIGURE 6. Mean serum antibody response to *C. jejuni* acid-extracted proteins following challenge.

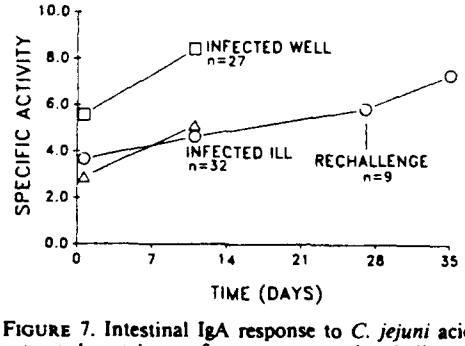

FIGURE 7. Intestinal IgA response to *C. jejuni* acid-extracted proteins on first exposure and rechallenge.

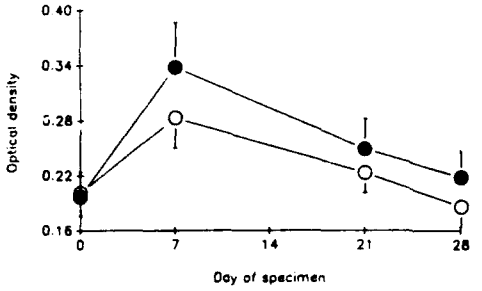

FIGURE 8. Serum IgA anti-LPS antibodies in 111 volunteers following challenge with *C. jejuni*. Symbols: ●, homologous challenge (n = 39); ○, heterologous challenge (n = 72).

response. There were no significant relationships between the severity of illness and the magnitude of the serologic response for any immunologic class.

Seven volunteers who became ill when fed strain 81-176 on initial challenge were rechallenged with the same strain 28 days later. Of these seven volunteers, five of whom became infected but none became ill, two had a rise in anti-LPS IgA and IgM levels after challenge; both of these persons had previously seroconverted after initial challenge.

OPSONIC ACTIVITY OF VOLUNTEER SERUM SAMPLES

To better understand the functional activity of the antibodies present in the serum samples from the volunteers, the ability of the sera to opsonize *C. jejuni* 81-176 was measured in a chemiluminescent (CL) assay (3, 4). To independently assess the effect of antibody and complement, serum samples were heated at 56°C for 30 min to inactivate complement, and a standard complement source from a hypogammaglobulinemic patient was added to each (Fig. 10). Normal human serum was a pool from three healthy persons uninfected with *C. jejuni* (2), and as a positive control, convalescent-phase serum from a volunteer infected with 81-176 who developed high IgG levels as measured by ELISA was used (1). All experiments were performed in triplicate on at least two separate days. The ratio of bacteria to neutrophils was 100:1.

In the absence of complement activity, antibodies present in normal human serum or in immune serum (data not shown) had no effect on CL response, and the complement source alone had a minimal effect. Adding back the complement source to the normal serum had little CL effect, but induced a strong CL response for the immune serum. In other experiments, the major activity of the immune serum was related to neutrophil ingestion of the organism, since pretreatment of the neutrophils with sodium fluoride blocked most of the CL response (data not shown).

Since homologous immune serum induced a strong response, the strain specificity of this response was assessed. Serum samples from the volunteers infected with strain A3249 (heterologous strain) were compared with those from the volunteers infected with strain 81-176 (homologous strain). In the presence of a complement source, as expected, the convalescent-phase serum to the homologous strain induced a strong

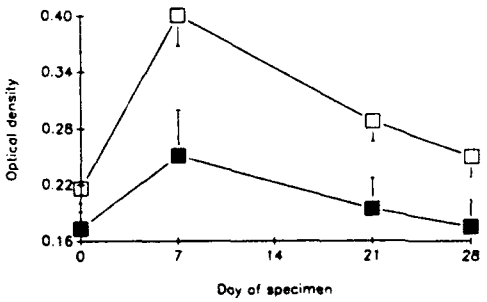

FIGURE 9. Serum IgA anti-LPS antibodies in well (■; n = 17) and ill (□; n = 22) volunteers infected with *C. jejuni* 81-176.

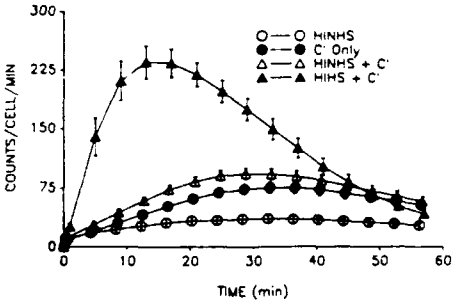

FIGURE 10. Polymorphonuclear cell CL response to *C. jejuni* 81-176 preopsonized with one of several serum sources. Abbreviations: HINHS, heat-inactivated normal human serum; C', complement source (serum sample from a hypogammaglobulinemic patient); HIHS, heat-inactivated immune human serum (convalescent-phase serum from a patient infected with strain 81-176).

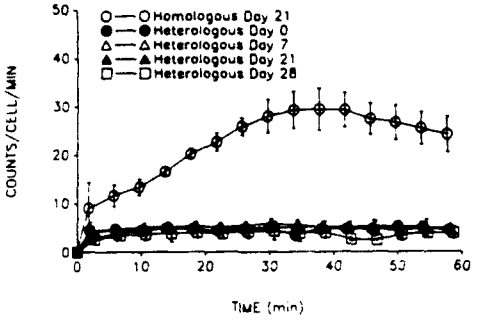

FIGURE 11. Polymorphonuclear cell CL response to *C. jejuni* 81-176 preopsonized with serum samples from volunteers challenged with strain 81-176 (homologous) or strain A3249 (heterologous). All serum samples were heat inactivated, and an exogenous complement source was added. Days refer to the number of days after *C. jejuni* challenge that serum samples were obtained.

response (Fig. 11), whereas serum samples from patients infected with the heterologous strain did not. These data suggest that infection induced strain-specific opsonizing activity.

To determine the kinetics of opsonizing activity in serum in the volunteers, serum samples from seven volunteers who were challenged (on day 0) and then rechallenged with strain 81-176 on day 28 were examined. Figure 12 shows the mean maximal values for the CL responses. Over the course of the first challenge, CL responses increased progressively until day 28. Upon rechallenge, which induced infection but not illness, CL responses declined. Therefore, homologous rechallenge did not boost the level of opsonizing activity in serum.

The data from the CL experiments indicate that the antibodies induced after *C. jejuni* infection not only recognize specific *C. jejuni* antigens, but also have opsonic activity. Such antibodies may play a role in vivo in permitting the elimination of extracellular *C. jejuni* within tissue. Normal serum apparently has little opsonizing activity, as does immune serum directed against the heterologous strain. The antigenic determinants inducing opsonizing activity are not known but are apparently strain specific. That homologous opsonizing activity rose progressively after *C. jejuni* challenge is not surprising; however, the lack of a booster response after rechallenge must be explained. One possibility is that the opsonizing antibodies are chiefly IgG and IgM, as they are after other infections. The development of effective gut immunity as measured by specific serum and intestinal IgA responses (as shown in Fig. 1 through 9) may have precluded tissue involvement that would lead to stimulation of IgG- or IgM-producing cells. Such a hypothesis has been proposed to explain the development of immunity to *C. jejuni* and falling specific IgG levels among children who are recurrently exposed to the organism under hyperendemic conditions in developing countries (2, 5).

CONCLUSIONS

Volunteer studies in healthy adults demonstrated that diarrheal (usually dysenteric) illnesses can be induced by ingestion of *C. jejuni*. With doses ranging from 8×10^2 to 2×10^9 CFU of two strains of *C. jejuni*, the rates of infection increased with the dose, but development of illness did not show a clear dose relationship. The resulting illness was more severe with strain 81-176 than with strain A3249. The dysenteric illness and presence of fecal leukocytes indicate that the pathogenesis of *C. jejuni* illness includes inflammation.

Protective immunity from prior disease was found when volunteers were rechallenged with the same strain 28 days later. Immunologic responses to acid-extracted proteins and to LPS after challenge with *C. jejuni* were assessed, and the opsonic activity of serum samples from the volunteers was evaluated by a CL assay, to better understand the functional activity of antibodies. Volunteers who became infected, but not those who remained uninfected, had a rise by day 7

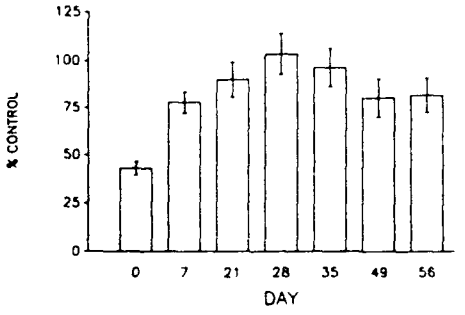

FIGURE 12. Polymorphonuclear cell CL response to *C. jejuni* 81-176 preopsonized with serum samples obtained from volunteers who were both challenged (day 0) and rechallenged (day 28) with strain 81-176. Data shown are mean (± standard error of the mean) maximal CL responses (counts per cell per minute) in comparison with a standard positive control.

in levels of serum IgA to acid-extracted proteins, and volunteers who became ill had higher peak levels. The serum immune response was greater for volunteers receiving strain 81-176. Infected volunteers also had a rise in intestinal *C. jejuni*-specific IgA levels. Volunteers with a high preexisting serum IgA level to acid-extracted proteins were less likely to become infected after challenge, and those with higher preexisting levels of intestinal IgA tended to remain well upon challenge. Volunteers who became infected had a rise in levels of serum IgA to LPS by day 7, and ill volunteers had higher peak levels. The serum immune response was greater against the homologous LPS (antigen from 81-176 used) than against the heterologous LPS. Postchallenge serum samples from volunteers induced a strong opsonizing activity, which appeared to be strain specific. It is likely that the antibodies induced by *C. jejuni* infection play a role by means of this opsonizing activity in eliminating the organism from tissue. Volunteer studies can be very useful for studying the serum and intestinal immune responses to *C. jejuni* infection and illness and can provide important insights into the extent and possible mechanisms of protection from prior exposure to *C. jejuni* or to artificial immunogens, such as candidate vaccines.

ACKNOWLEDGMENTS. We thank Paul F. Smith for technical assistance and John Repine for assistance with the CL studies. The work described in this chapter was supported in part by an interagency agreement between the U.S. Army Medical Research and Development Command and the Department of Veterans Affairs, by the Thrasher Research Fund, and by contract AI-12666 from the National Institute of Allergy and Infectious Diseases.

REFERENCES

1. **Black, R. E., M. M. Levine, M. L. Clements, T. P. Hughes, and M. J. Blaser.** 1988. Experimental *Campylobacter jejuni* infection in humans. *J. Infect Dis.* 157:472–479.
2. **Blaser, M. J., R. E. Black, P. F. Duncan, and J. Amer.** 1985. *Campylobacter jejuni*-specific serum antibodies are elevated in healthy Bangladeshi children. *J. Clin. Microbiol.* 21:164–167.
3. **Blaser, M. J., P. A. Smith, J. E. Repine, and K. A. Joiner.** 1988. Pathogenesis of *Campylobacter fetus* infections. Failure to bind C3b explains serum and phagocytosis resistance. *J. Clin. Invest.* 81:1434–1444.
4. **Blaser, M. J., P. F. Smith, and P. A. Kohler.** 1985. Susceptibility of *Campylobacter* isolates to the bacterial activity in human serum. *J. Infect. Dis.* 151:227–235.
5. **Blaser, M. J., D. N. Taylor, and P. Echeverria.** 1986. Immune response to *Campylobacter jejuni* in a rural community in Thailand. *J. Infect. Dis.* 153:249–254.
6. **Blaser, M. J., D. N. Taylor, and R. A. Feldman.** 1983. Epidemiology of *Campylobacter jejuni* infections. *Epidemiol. Rev.* 5:157–176.
7. **Calva, J. J., G. M. Ruiz-Palacios, A. B. Lopez-Vidal, A. Ramos, and R. Bojalil.** 1988. Cohort study of intestinal infection with *Campylobacter* in Mexican children. *Lancet* i:503–506.
8. **Levine, M. M., R. E. Black, M. L. Clements, C. Lanata, S. Sears, T. Honda, C. R. Young, and R. A. Finkelstein.** 1984. Evaluation in humans of attenuated *Vibrio cholerae* El Tor Ogawa strain Texas Star-SR as a live oral vaccine. *Infect. Immun.* 43:515–522.
9. **Lior, H.** Personal communication.

Campylobacter jejuni

Current Status and Future Trends

Editors

Irving Nachamkin
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania 19104

Martin J. Blaser
Vanderbilt University School of Medicine
and Department of Veterans Affairs Medical Center
Nashville, Tennessee 37232

Lucy S. Tompkins
Stanford University School of Medicine
Stanford, California 94305

American Society for Microbiology
Washington, D.C.